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The 5-HT7 receptor antagonist SB-269970 alleviates seizure activity and downregulates hippocampal c-Fos expression in pentylenetetrazole-induced kindled rats

Bilal Sahin[a], Ercan Ozdemir[a], Erkan Gumus[b], Mustafa Ergul[c] and Ahmet Sevki Taskiran[a]

[a]Departments of Physiology, Sivas Cumhuriyet University, School of Medicine, Sivas, Turkey; [b]Departments of Histology and Embryology, Aydın Adnan Menderes University, School of Medicine, Aydın, Turkey; [c]Departments of Biochemistry, Sivas Cumhuriyet University, School of Pharmacy, Sivas, Turkey

ABSTRACT

Objectives: Recently, studies have demonstrated that serotonin type 7 receptors (5-HT7) have conflincting effects on neuronal excitability in different brain regions. However, the effect of 5-HT7 on seizures has not been exactly elucidated yet. Therefore, our aim in this study was to investigate the effects of 5-HT7 antagonist SB-269970 on pentylenetetrazole (PTZ) induced fully kindled rats.

Methods: In the study, 32 adult male Wistar Albino rats (weighing 220–260 g) were used. Rats were injected with PTZ (35 mg/kg) intraperitoneally every other day to generate kindling model. 5-CT (0.1 mg/kg) and SB-269970 (1 mg/kg) were administered 30 min before acute seizure induction with PTZ (35 mg/kg). Seizure stages were determined according to the Racine scale. After electrocorticography (ECoG) recordings of seizure-induced rats were obtained, the animals were sacrificed by decapitation. The hippocampal GABA levels were determined by ELISA kit and the number of c-Fos positive neurons in the hippocampal dentate gyrus (DG), CA1 and CA3 areas were measured by immunohistochemical method.

Results: The results showed that SB-269970 reduced the number of spikes, percent seizure duration and duration of generalized tonic-clonic seizures (dGTCS), while increasing the onset time of generalized tonic-clonic seizures (oGTCS). The hippocampal GABA levels were significantly increased in the SB-269970 group compared with the PTZ group. In addition, SB-269970 reduced the number of c-Fos positive cells in hippocampal CA1 area.

Discussion: 5-HT7 antagonist SB-269970 displays anticonvulsant effects on PTZ-induced seizures in fully kindled rats and these effects may be related to GABAergic activity in the hippocampus.

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5-HT receptor; 5-HT7; SB-269970; seizure; c-Fos; GABA

Introduction

Epilepsy is one of the most common serious neurological disorders in the world characterized by sudden and repetitive seizures [1]. It affects more than 50 million people worldwide and causes significant morbidity and mortality [2]. Seizures are defined as abnormal neuronal firing that can lead to motor, sensory, autonomic, or psychological clinical changes in neurological function [3]. Recurrent seizures can transform a normal brain into an epileptic brain by undergoing a series of cellular-molecular, structural and/or functional changes, giving it the ability to produce permanent and spontaneous seizures [4]. In this process, changes in gamma-aminobutyric acid (GABA), which is the primary inhibitor of synaptic mechanisms, and glutamate systems, which are the primary exciters, are mostly responsible [5].

Serotonin, also known as 5-hydroxytryptamine (5-HT), and its receptors are now firmly established to be involved in various types of seizures [6]. Most studies indicate that increased synaptic 5-HT levels using selective serotonin reuptake inhibitors (SSRIs) prevents focal and generalised seizures, while decreased level of 5-HT in the brain lowers the threshold for audiogenic, chemical and electrical seizures [7,8]. Serotonergic receptors are divided into seven different groups as 5-HT1 to 5-HT7 and 14 different subgroups according to their structure, function and location [9]. The 5-HT7 receptor is a G-protein-coupled receptor and is strongly expressed in the brain, particularly the thalamus, the hypothalamus, the hippocampus, and cortex [10]. With the genetic deletion of these receptor subtype and the increased use of specific agonists and antagonists, it has been possible to investigate the role of 5-HT7 receptor in seizures. In this context, 5-HT7 receptor were described as having a role in epilepsy and/or the spread of seizures [11]. Administration of SB-258719, a less potent 5-HT7 antagonist than SB-269970 [12], has

CONTACT Bilal Sahin bilalsahin@cumhuriyet.edu.tr Department of Physiology, School of Medicine, Sivas Cumhuriyet University TR-58140, Sivas, Turkey

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been shown to reduce spontaneous seizure activity in the absence epilepsy [13]. However, in 5-HT7 receptor knockout mice, lower electrical and chemical seizure thresholds has been observed [14].

Animal models of epilepsy and epileptic seizures are crucial to understanding the basic mechanisms involved in epileptogenesis. Pentylenetetrazole (PTZ) is a selective antagonist of $GABA_A$ chloride ionophore complex and stimulates epileptogenic activity by inhibiting GABA-mediated transmission [15]. Anatomically, it has been shown that the 5-HT7 receptor is expressed in all subfields of the hippocampus, especially in the CA3 and dentate gyrus regions [16]. Moreover, evidence show that 5-HT7 receptors modulate GABAergic transmission in rat hippocampal CA1 area [17]. These data suggest that GABAergic activity may play a role in the effects of the 5-HT7 receptor on seizure control. Besides, in the rat hippocampus, PTZ increased the expression of c-Fos, an immediate early gene, which is an indicator of increased neuronal activity [18]. Therefore, the aim of this study was to investigate the effect of SB-269970, a 5-HT7 antagonist, on seizure parameters, c-Fos expression and GABA levels in hippocampus in PTZ-induced fully kindled rats.

Materials and methods

Animals

Thirty-two adult male 8–13 weeks old Wistar albino rats (weighing 220–260 g) were used in this study. Prior to the study, permission was obtained from Cumhuriyet University Animal Experiments Local Ethics Committee (No: 2016/96) [19]. Rats were obtained from the Experimental Animals Laboratory of Sivas Cumhuriyet University and kept at 22 ± 1 °C room temperature for 12 hours in a light/dark cycle, in a sound insulated room and containing 55 ± 6% humidity and fed in an appropriate ratio. Experimental studies were carried out between 09.00 and 12.00 every day in accordance with the circadian rhythm changes. In addition, the light and sound levels of the test medium were kept under constant control. Throughout the study, efforts were made to minimize the suffering, and to use the minimal number of animals.

Drugs

Before experimental application Pentylenetetrazole (PTZ), 5-Carboxamidotryptamine (5-CT, 5-HT7 agonist) and SB-269970 (5-HT7 antagonist) dissolved in saline. All agents were administered intraperitoneally. All research drugs were purchased from Sigma-Aldrich (Co. St Louis, MO).

PTZ kindling

PTZ-kindling was carried out as described in previous studies [20]. PTZ (35 mg/kg) was administered intraperitoneally to all animals on every Monday, Wednesday, and Friday up to 15 injections. Following each injection, animals were observed over a 30-min period. Rat seizure activity was scored using the revised Racine scale [21]. Stage 0: No response, Stage 1: Ear and facial twitching, Stage 2: Head nodding, Stage 3: Myoclonic jerks, Stage 4: Tonic–clonic seizures without loss of postural control, Stage 5: Tonic–clonic seizures with loss of postural control, Stage 6: Tonic–clonic seizures with wild running and jumping, and Stage 7: Lethal seizure. The study was continued with kindled rats that reached stage 4 or stage 5 seizures following three consecutive injections of PTZ. However, animals that died or did not reach stage 4 or 5 seizures during the kindling process were excluded from the study (n = 4).

Experimental design

Following kindling procedure, rats were placed electrodes to obtain electrocorticography (ECoG) recordings and rested for one-week recovery period. Subsequently, rats were randomly divided into 4 groups (n = 7). Control group: Rats received normal saline (1 ml/kg, i.p.) every other day (EOD), for a total of 21 days, PTZ group: Rats received PTZ (35 mg/kg, EOD, i.p.) for 21 days and injected with normal saline (1 ml/kg, i.p.) 30 min before acute PTZ (35 mg/kg) injection, 5-Carboxamidotryptamine group: Rats received 5-CT (0.1 mg/kg, i.p.) 30 min before PTZ (35 mg/kg) injection and SB-269970 group: Rats received SB-269970 (1 mg/kg, i.p.) 30 min before PTZ (35 mg/kg) injection (Figure 1). Drug doses were determined according to previous studies [22,23]. Following acute PTZ injections, animals were placed in open transparent plexiglass cages (50x40x40 cm in diameter). Animal behaviour was observed and ECoG activity was recorded for 30 minutes following each injection, and the seizure stages were determined based on the Racine scale (RS). After completion of the experimental process, all experimental animals were sacrificed by decapitation. Right- and left-brain hemispheres seperated. Right-brain tissue was removed and centrifuged following homogenization for GABA analysis in each sample and frozen at −80°C. Left-brain tissue samples reserved for histological examination were stored in neutral buffered formaldehyde.\

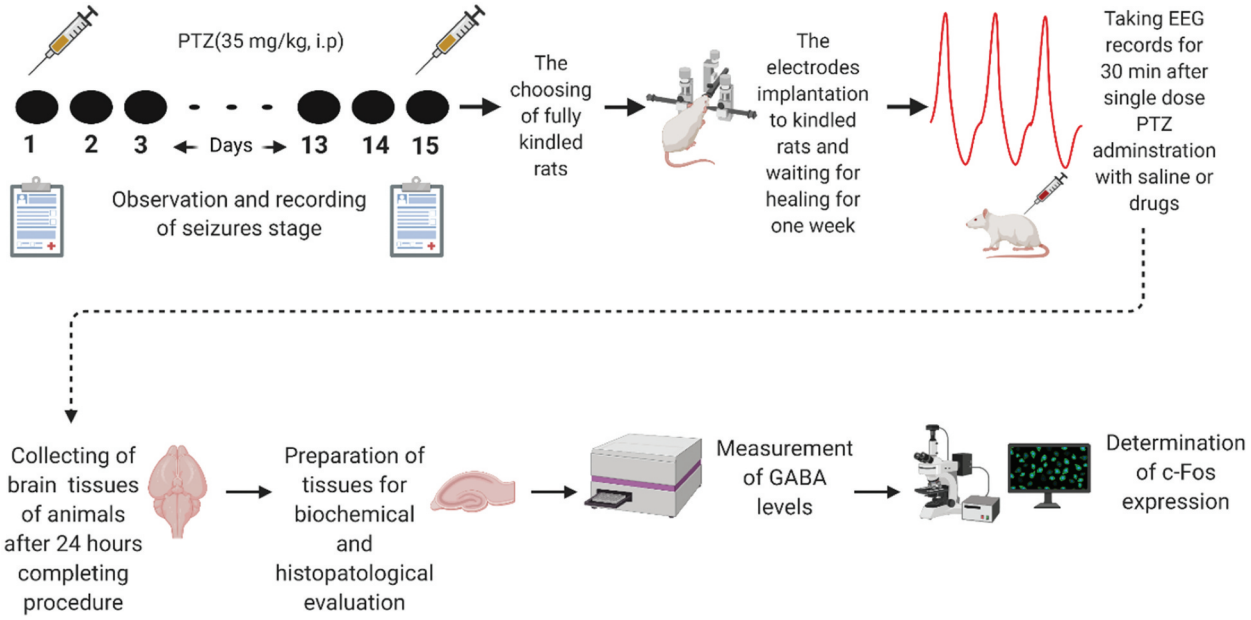

Figure 1. Experimental design of the study (Created with BioRender.com).

Stereotaxic surgery

The animals were injected with ketamine (90 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) to provide anesthesia before the administration. The depth of anesthesia was controlled with corneal and paw reflexes. After the hairs on the scalp were shaved, the rat was placed on the stereotaxy device for surgical procedures so that the bregma and lambda points were in the same plane. Approximately 3 cm incision was made in the scalp of the animal with a lancet. The bone tissue was reached by removing the tendons and fascia under the scalp. Bleeding in the soft tissue was prevented by packing with gauze. The locations of the screws where the electrode will be placed were calculated using the rat brain atlas of Paxinos and Watson (1998), and the bregma was referenced and determined as the '0' point. Three different small burr holes were made with a drill (diameter 1 mm) at the points determined in the skull of the rat. A stainless steel screw was placed in these holes in contact with the brain membranes for ECoG recording. Tripolar electrodes were placed over the motor cortex. Electrode coordinates were determined as in our previous study [1]. All of the electrodes (0.12 mm diameter, Plastic One, Roanoke, VA, USA) were fixed to the skull with two layers of dental acrylic and linked by insulated wires to a connector for the ECoG recordings. After this procedure, the animals were injected with 50 mg/kg sultamicillin (i.p.) twice a day for 3 days to prevent infection. Rats were allowed to recover for one week before the initiation of the experimental studies.

Electrocorticography recordings

After the recovery period (7-days), animals were placed individually in plexiglass cages for 30 min for habituation. The frequency and amplitude of the ECoG activity were measured off-line. ECoG recordings were filtered between 1 and 34 Hz to eliminate ambient noise signals using PowerLab 4/SP (Figure 2). Drugs were administered 30 min before 35 mg/kg PTZ injection to induce seizures. ECoG recordings were analyzed using LabChart (v7.0.3).

Seizure stage, onset time of first myoclonic jerk (FMJ, time from injection to first spike), the onset of generalized tonic–clonic seizures (oGTCS), the duration of generalized tonic–clonic seizures (dGTCS) were calculated based on video and ECoG recordings during 30 min. The number of spikes per minute and the percentage of seizure duration were calculated. Spike detection algorithm was used considering three parameters of spikes (shape, amplitude and duration) [24]. The seizure times corresponding to epileptic spikes for 30 minutes were calculated using the program and converted to percentages, and referred to as the percentage of seizure duration. ([Seizure time * 100]/1800; 1800 = seconds, corresponding to 30 minutes) [25].

GABA level measurement

Animals decapitation, hippocampus was isolated from brain tissue. Extracted brain tissue was stored in a freezer (−80 °C). For the brain tissue homogenization, 50 mM Tris-HCL (pH: 7.5) buffer was used and transferred to glass tubes under

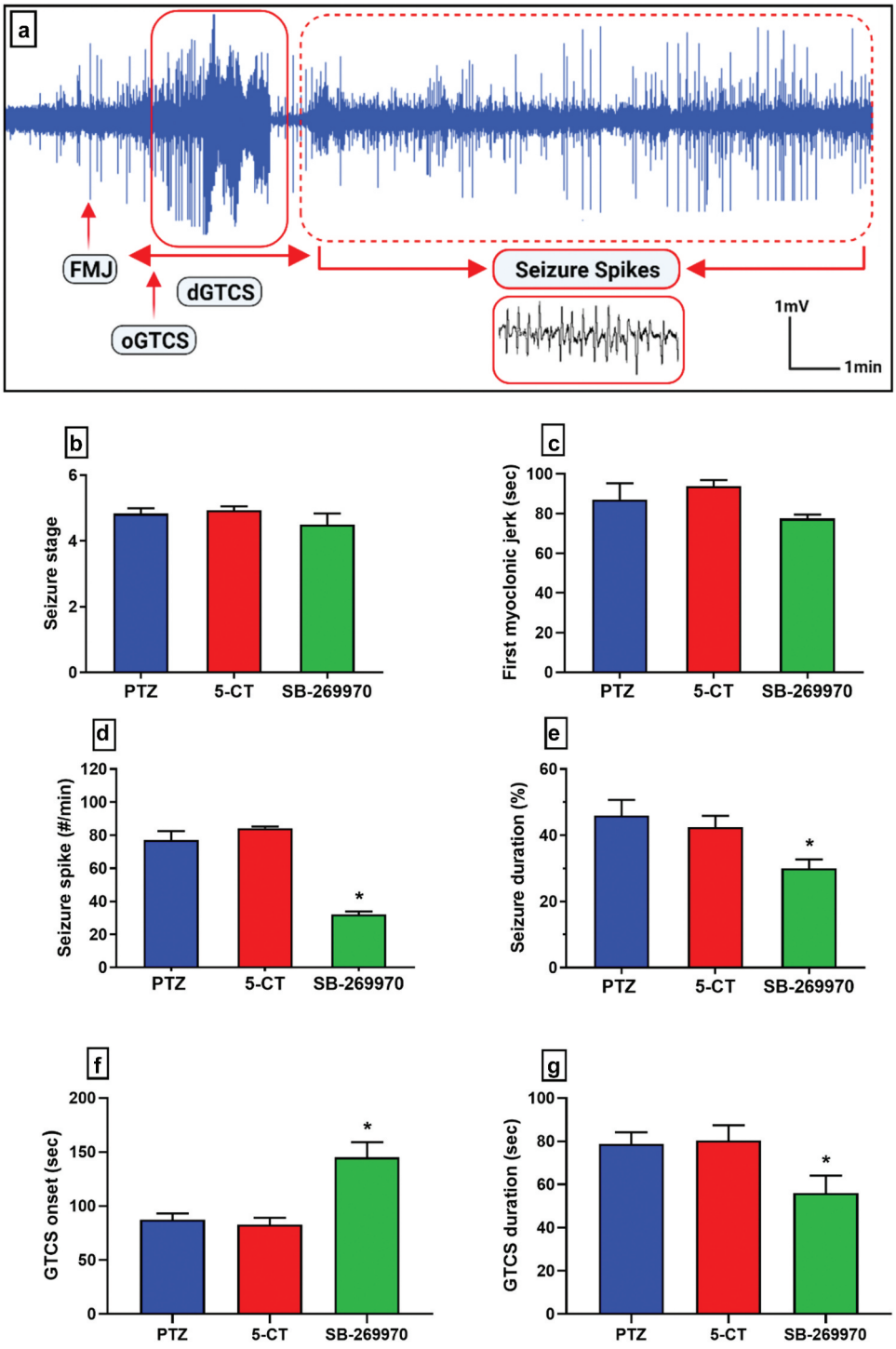

Figure 2. Effects of drugs on seizures. The example of ECoG recordings for 30 min. The effects of 5-CT and SB-269970 on seizure stage (b), onset time of FMJ (c), spike number (per min.) (d), percentage of seizure duration (E), generalized tonic clonic seizure onset time (oGTCS) (f) and generalized tonic-clonic seizure duration (dGTCS)(g). *p < 0.05, compared to the PTZ group.

cold-chain conditions. 2 ml Tris-HCL buffer was added to the tissue sample. Tissue samples were placed in Eppendorf tubes and homogenized for 3 minutes at 16,000 rpm. The buffer was added at 5 times the weight of the compound. Centrifuged supernatants from each sample were used to measure GABA levels in the hippocampus using a GABA ELISA kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) as specified in the manufacturer's instructions.

Immunohistochemical analysis

Tissue samples of left cerebral hemispheres were fixed in 10% neutral buffered formalin for 30–36 hours and were stored to +4 °C after dehydration, transparency and paraffin embedding. After the blocks were kept in – 20°C for a few hours, 4 µm thick sections were cut with a microtome (Leica RM2235, Nussloch, Germany). Each 12th section of serial sections was taken on a poly-L-lysine coated slide for evaluation, and 5 sections for each rat were evaluated for c-Fos

immunoreactivity. Briefly, following deparaffinization in xylene and rehydration with graded series of ethanol, the sections were blocked using 3% hydrogen peroxide in methanol for 15 min at RT. After washing in phosphate buffered saline with Tween-20 (PBS-Tween-20), antigen retrieval was performed on the sections by boiling twice for 10 min in 10mMsodium citrate buffer (pH 6). The sections were incubated for 10 min in Ultra V Block (Thermo Scientific, USA) at RT to prevent non-specific binding, and then treated with anti-c- Fos antibody (1:100, sc-166,940, Santa Cruz) overnight at +4 °C. After washing, these sections were incubated with HRP-streptavidin labeled, a biotinylated secondary antibody (TP-015-HA; Thermo Scientific, USA) and visualized with DAB (3,3′-Diaminobenzidine) and counterstained with hematoxylin. All the steps of the immunohistochemistry staining processes were applied to the negative control sections without the primary antibody incubation. Hippocampal CA1, CA3 and dentate gyrus (DG) were examined at 400X magnification by light microscopy (Olympus BX5, Olympus corp. Tokyo, Japan). The c-Fos positive neurons in each region of hippocampus (CA1, CA2, CA3 and DG) were dyed brown by DAB-IHC staining and counted manually by their morphological features, using NIH ImageJ software (National Institutes of Health, Bethesda, MD). The number of total cells in each region of hippocampus were acquired by nuclear staining with hematoxylin. The percentage of c-Fos positive neurons equals to brown cells/total cells [26].

Statistical analysis

Statistical analysis of numeric data from electrophysiological records was conducted using the SPSS program (SPSS 22.0 for Windows, Chicago, IL, USA). Data are expressed as mean ± SEM. Normal distribution was assessed according to the Shapiro Wilk test. One-way analysis of variance (ANOVA) followed by Tukey's HSD multiple comparisons was used for data analysis. Statistical significance level was accepted as $p < 0.05$.

Results

The effects of *SB-269970* and 5-CT on seizures

After the kindling model was established (by administering PTZ (35 mg/kg) 15 times every other day), kindled rats (stage 4 or 5 seizures after three consecutive PTZ injections) were randomly divided into 3 groups, and the acute received vehicle (PTZ), 5-CT (0.1 mg/kg) and SB-269970 (1 mg/kg) for 30 min before PTZ (35 mg/kg) to test the anticonvulsant effect (Figure 2A).

Depending on the seizure stage, the means of seizure stages were 4.83 ± 0.16 in the PTZ group, 4.83 ± 0.16 in the 5-CT group and 4.50 ± 0.34 in the SB-269970 group. There was no statistically significant difference between the groups ($p > 0.05$; Figure 2B).

The mean values of FMJ onset time as seconds were 87.00 ± 8.22 in the PTZ group, 93.75 ± 3.16 in the 5-CT group and 77.50 ± 1.92 in the SB-269970 group. There was no statistically significant difference between the groups depending on FMJ onset time ($p > 0.05$; Figure 2C).

The means of spike frequency (seizure spikes for per minute during 30 min) were 77.11 ± 5.37 in the PTZ group, 87.8 ± 8.3 in the 5-CT group and 32.18 ± 1.75 in the SB-269970 group. SB-269970 significantly decreased spike frequency compared with PTZ and 5-CT group ($p < 0.01$, Figure 2D)

The means of seizure duration were 46.00 ± 4.66 in the PTZ group, 42.44 ± 3.44 in the 5-CT group and 29.99 ± 2.71 in the SB-269970 group. SB-269970 significantly decreased seizure duration compared with the PTZ group ($p < 0.05$). However, 5-CT slightly decreased seizure duration compared with PTZ, but it was not statistically significant ($p > 0.05$; Figure 2E).

The means of GTCS onset were 87.28 ± 5.82 in the PTZ group, 82.50 ± 6.80 in the 5-CT group and 145.00 ± 14.00 in the SB-269970 group. SB-269970 significantly increased GTCS onset time compared with the PTZ group ($p < 0.05$). On the other hand, 5-CT slightly decreased GTCS onset time compared with PTZ, but it was not statistically significant ($p > 0.05$; Figure 2F).

The means of GTCS duration were 78.71 ± 5.44 in the PTZ group, 80.33 ± 7.05 in the 5-CT group and 56.00 ± 8.10 in the SB-269970 group. SB-269970 significantly decreased GTCS duration compared with the PTZ group ($p < 0.05$). Besides, 5-CT slightly increased GTCS duration compared with PTZ, but it was not statistically significant ($p > 0.05$; Figure 2G).

The effect of *SB-269970 and* 5-CT on hippocampal GABA levels

The means of GABA levels in the brain were 139.94 ± 4.57 in the control group, 98.97 ± 6.99 in the PTZ group, 106.76 ± 5.48 in the 5-CT group and 138.99 ± 9.57 in the SB-269970 group. PTZ decreased brain GABA levels compared with control group ($p < 0.05$). On the other hand, SB-269970 increased brain GABA levels significantly compared with the PTZ group ($p < 0.05$). However, there was no statistically significant difference between 5-CT and PTZ groups ($p > 0.05$, Figure 3).

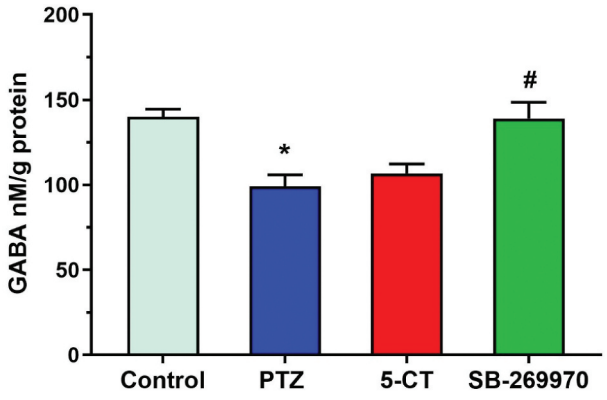

Figure 3. Hippocampal GABA levels. *p < 0.01; compared to the control group. #p < 0.05; compared to the PTZ group.

The effect of SB-269970 and 5-CT on post-seizure c-Fos immunoreactivity in hippocampal CA1, CA3 and DG regions

The effect of SB-269970 and *5-CT on post-seizure c-Fos immunoreactivity in hippocampal CA1 area*

As shown in Figure 4, PTZ increased c-Fos positive neurons in the CA1 region compared with the control group ($p < 0.001$). On the other hand, both 5-CT and SB-269970 significantly reduced c-Fos immunoreactivity compared with the PTZ group ($p < 0.001$). Moreover, the reduction of c-Fos positive neurons was significantly higher in the 5-CT group compared with the SB-269970 group ($p < 0.05$; Figure 4).

The effect of SB-269970 and *5-CT on post-seizure c-Fos immunoreactivity in hippocampal CA3 area*

As shown in Figure 6, PTZ increased c-Fos positive neurons in the CA3 region compared with the control group ($p < 0.001$). Conversely, 5-CT reduced c-Fos positive neurons compared with the PTZ group ($p < 0.01$). SB-269970 slightly increased c-Fos positive neurons, but it was not statistically significant ($p > 0.05$; Figure 5).

3.3.3 The effect of SB-269970 and *5-CT on post-seizure c-Fos immunoreactivity in hippocampal DG area*

As also shown in Figure 6, PTZ increased c-Fos positive neurons in the DG region compared with the control group ($p < 0.001$). Moreover, SB-269970 significantly increased c-Fos positive neurons compared with the PTZ group ($p < 0.01$). In contrast, 5-CT decreased c-Fos positive neurons significantly compared with the PTZ group ($p < 0.01$; Figure 6).

Discussion

In this study, we attempted to assess the effect of 5-HT7 antagonist SB-269970 on epileptic seizures, hippocampal GABA levels and hippocampal c-Fos

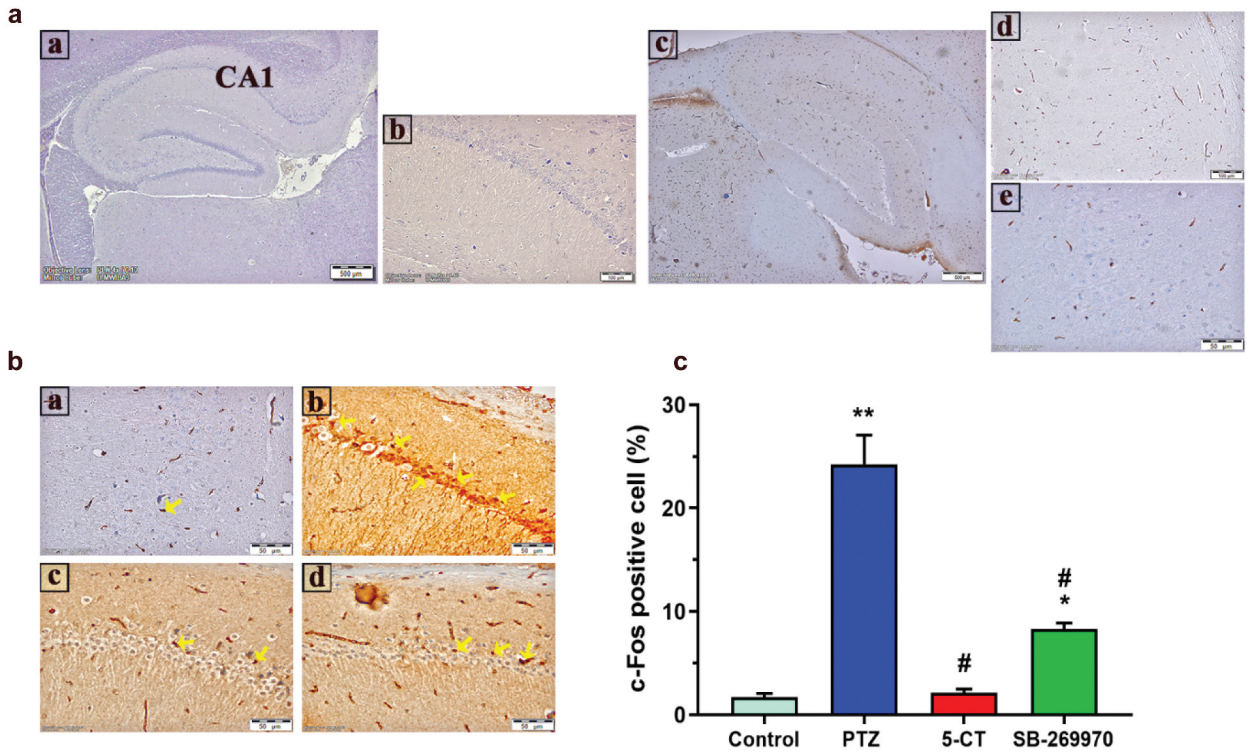

Figure 4. Effect of drugs on c-Fos expression in the hippocampal CA1 area. (a) Control group light microscopic image (a, X40 and b, X100) and c-fos immunohistochemistry (c, X40; d, X100 and e, X100 negative control) image. (b) c-Fos immunoreactivity (X400). a, Control; b, PTZ; c, 5-CT; d, SB-269970; (c) Number of c-fos positive neurons in hippocampal CA1 area. *p < 0.01, **p < 0.001; compared to the control group. #p < 0.001; compared to the PTZ group. ↑ (yellow arrow), sample c-Fos positive neurons.

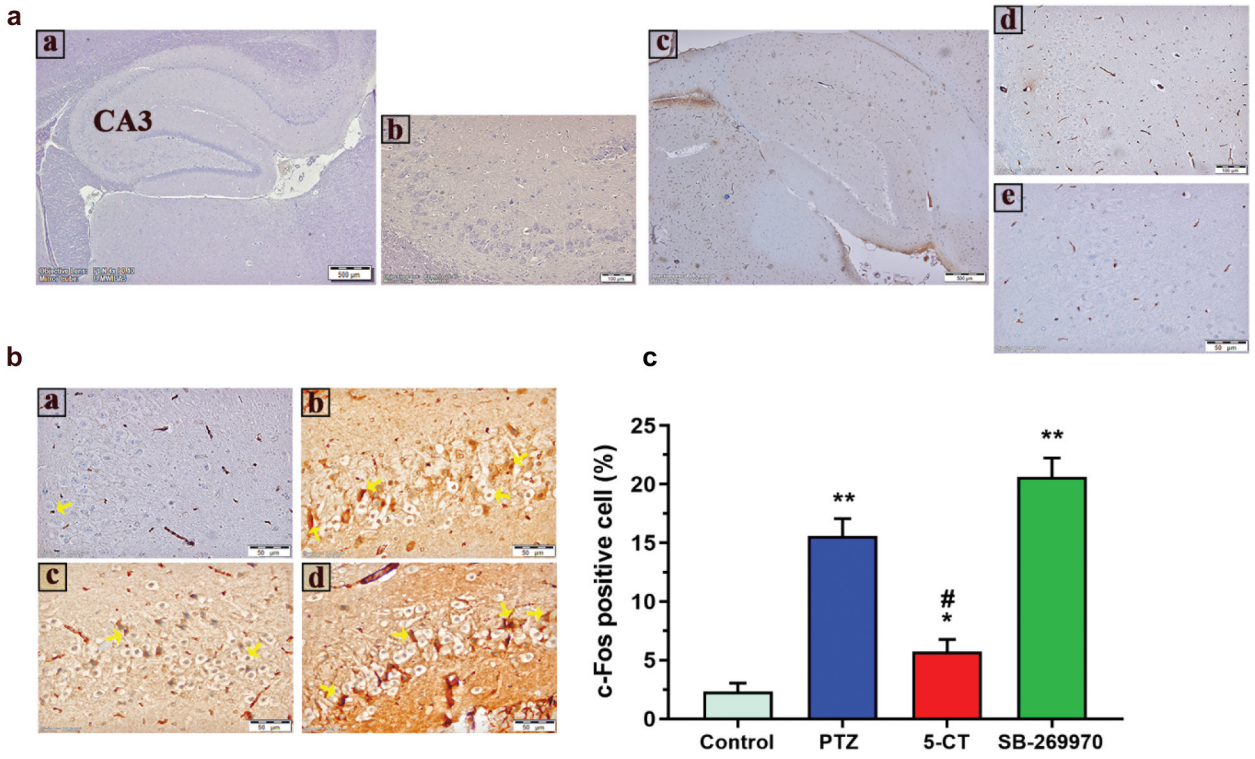

Figure 5. Effect of drugs on c-Fos expression in the hippocampal CA3 area. (A) Control group light microscopic image (a, X40 and b, X100) and c-fos immunohistochemistry (c, X40; d, X100 and e, X100 negative Control) image. (B) c-Fos immunoreactivity (X400). a, Control; b, PTZ; c, 5-CT; d, SB-269970; (C) Number of c-fos positive neurons in hippocampal CA3 area. *$p < 0.01$, **$p < 0.001$; compared to the control group. $^{\#}p < 0.01$; compared to the PTZ group. ↑ (yellow arrow), sample c-Fos positive neurons.

expression in PTZ-kindling model of epilepsy. Our findings showed that SB-269970 decreased the spike number, the percentage of seizure duration and dGTCS and increased the oGTCS. However, 5-CT did not show any significant effect on seizure stage and ECoG activity. GABA levels in the PTZ group was significantly lower than the control group. In contrast, SB-269970 increased GABA levels compared with the PTZ group. Moreover, PTZ increased c-Fos positive cells in all hippocampal areas. In addition, SB-269970 decreased c-Fos positive cells in hippocampal CA1 area.

5-HT7 receptor is a membrane bound Gs protein-dependent receptor expressed primarily in the dorsal raphe nuclei (DRN), thalamus, hypothalamic hippocampus and gastrointestinal tract. The DRN is the major cause of extensive 5HT innervation that regulates the activity of neural networks of forebrain targets via multiple 5HT receptor subtypes [27]. Activation of 5HT7 receptors increases the average frequency of spontaneous inhibitory postsynaptic currents (sIPSCs) recorded from DRN-projecting cells, induces hyperpolarization, and reduces firing frequency [28]. Besides, SB269970 normalized the frequency of sIPSCs in DRN projection cells o and restored the reactivity of 5-HT7 receptors in the DRN [29]. Possibly due to differences in epilepsy patterns, the 5-HT7 receptor mediated effects are heterogeneous and controversial. Unlike a few studies, many studies support a proconvulsant role for 5-HT7 receptor [6,13,30–33]. 5-HT7 agonist, 5-CT, increases blast discharges in the hippocampal CA3 area in rats. The abolition of this increase by administration of SB-269970 indicates that inhibition of 5-HT7 receptor decreases the activity of CA3 neurons in the hippocampus [31]. In a rat model of pilocarpine-induced temporal lobe epilepsy (TLE), 5-HT7 agonist AS19 increased the frequency of spontaneous recurrent seizures and spike wave discharges. Conversely, in the same study, SB269970 had an inhibitory effect on seizures [34]. In addition, Gill et al. showed that expression of 5-HT7 receptor in the cerebral cortex, thalamus, hippocampus and amygdala in rats with spontaneous recurrent seizures was significantly higher than in control animals [31]. Furthermore, SB-269970 was found to completely suppress the anticonvulsant properties of 5-CT in a swimming stress test in mice [23]. However, 8-OH-DPAT, a 5HT1/5HT7 agonist, had showed no effect on amigdala kindling [35], electroshock and PTZ-induced seizures [36] in rats. In line with these studies, in our study, 5-HT7 agonist 5-CT had no effect on seizures. A few studies suggesting an anti-convulsive role of 5-HT7 receptor activation against seizures. In a PTZ-induced seizure study, 5-CT have been shown to increase serotonin levels in the anterior thalamus of rats and increased FMJ onset time [37]. The differences between our study and these studies may be

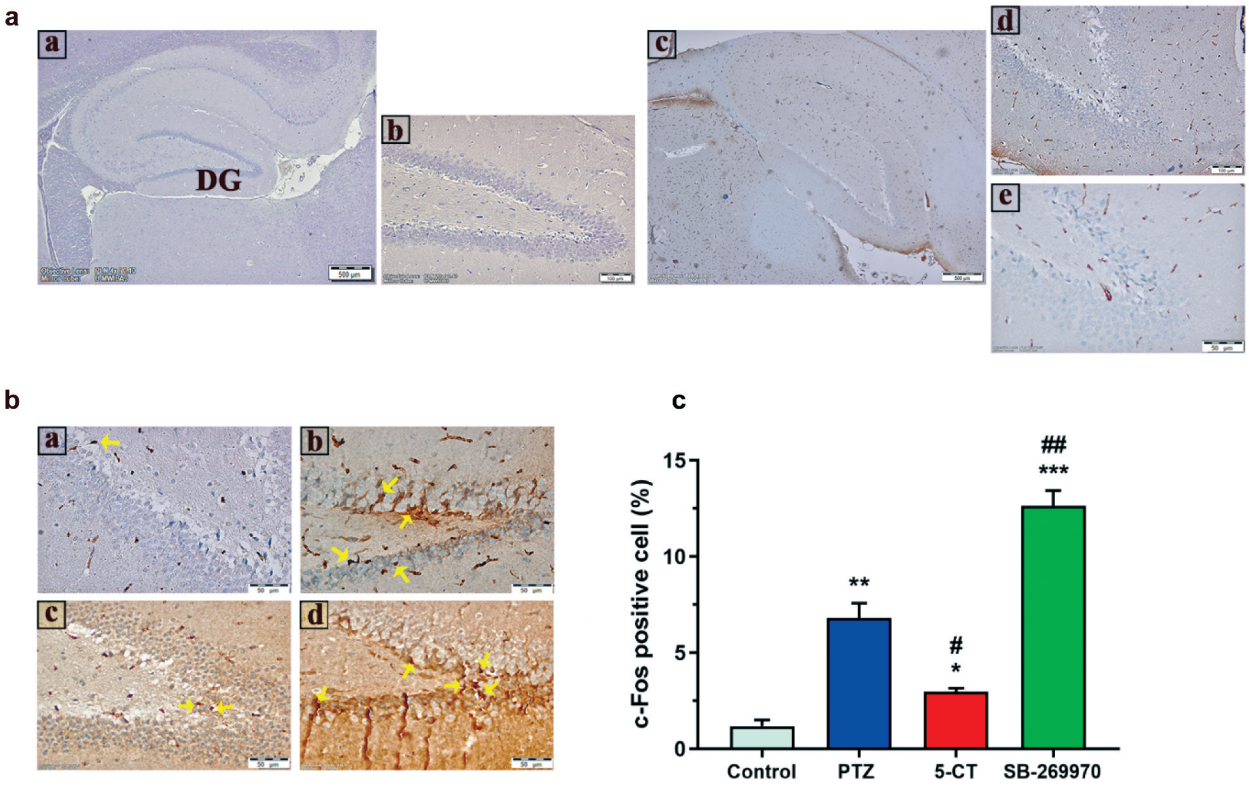

Figure 6. Effect of drugs on c-Fos expression in the DG area. (A) Control group light microscopic image (a, X40 and b, X100) and c-fos immunohistochemistry (c, X40; d, X100 and e, X100 negative control) image. (B) c-Fos immunoreactivity (X400). a, Control; b, PTZ; c, 5-CT; d, SB-269970; (c) Number of c-fos positive neurons in DG. *p < 0.05, **p < 0.01, ***p < 0.001; compared to the control group. $^{\#}$p < 0.01, $^{\#\#}$p < 0.001; compared to the PTZ group. DG, dentate gyrus. ↑ (yellow arrow), sample c-Fos positive neurons.

related to the diversity in the epilepsy models. In addition, 5-HT7 knockout mice were found to be more susceptible to electroshock-induced tonic seizures and PTZ-induced convulsions [14]. Moreover, one study reported that 5-HT7 modulation had no effect on seizure thresholds. In this study, the selective 5-HT7 antagonist SB-258741 did not alter seizure thresholds in MES and PTZ mice epilepsy model [38]. The apparent contradiction between this effect and the provoking effects of 5-HT7 activation may be associated with adaptive changes in the neuronal circuits of 5-HT7 knockout animals.

GABA, the main inhibitory neurotransmitter of the central nervous system, mainly acts on the $GABA_A$ receptor [39]. Biochemical and electrophysiological studies have shown that the pharmacological effects of PTZ are induced by blocking the binding sites of the $GABA_A$ receptor complex, known as benzodiazepine recognition sites [40]. Hippocampal GABA levels and number of GABAergic interneurons decreased in PTZ-kindled rats [41,42]. Moreover, Kaura et al. reported a reduction in extracellular GABA levels in kindled rat amygdala [43]. Also, GABA levels in cerebrospinal fluid of patients with epilepsy has been found to be lowered in early studies [44–46]. In a recent study in PTZ-kindling model epilepsy in rats, Taskiran et al. showed that GABA levels in the brain decreased after PTZ-induced seizures. In consistent with their study, in the present study, GABA levels were higher in the PTZ kindled rats [1]. 5-HT7 receptors are involved in serotonergic and GABAergic interactions in certain brain structures. 5-HT7 receptors in the DRN are localized on local GABAergic interneurons, which modulate the activity of 5-HT projection neurons. Thus, the activation of 5-HT7 receptors affects 5-HT release by modulating GABAergic transmission in DRN [47]. Moreover, 5-HT7 receptor activation inhibits GABAergic interneurons that target serotoninergic neurons in the raphe nucleus, and then increases 5-HT release in other areas of the brain [48]. On the other hand, 5-HT7 receptors decrease GABA-dependent currents in neurons of the hypothalamic suprachiasmatic nucleus [49]. Tokarski et al. showed that, in normal rat brain, activation of 5-HT7 receptors have enhanced the frequency of GABA-mediated sIPSCs in hippocampal CA1 neurons [17]. Similarly, in a normal rat globus pallidus, 5-HT7 receptor activation increased GABAergic transmission. However, in the present study, 5-HT7 antagonist SB-266970 increased hippocampal GABA levels. This contrast may be due to changes in brain neuronal circuits in kindling model epilepsy. Moreover, SB-266970 also decreased the spike number and percentage seizure duration, 5-HT7 receptor antagonists may show anti-convulsive properties via GABA mediated mechanism.

c-Fos is an immediate early response gene implicated in cellular proliferation and differentiation following extracellular stimuli. c-Fos is currently used as a marker for neuronal activity and has been linked to a number of neuronal and behavioural responses to acute expression of stimuli [50]. Many studies have shown that PTZ significantly increases c-Fos expression in many brain regions, especially in the hippocampus [51,52]. Repeated administration of PTZ at subconvulsive doses induced clonic seizures and status epilepticus, while increasing c-Fos expression in the hippocampal dentate gyrus, striatum, and nucleus accumbens in adult rats. During the avaluation of PTZ- kindling seizures, c-Fos expression increased in the basolateral amygdala and CA1 region of the hippocampus during the fourth stage of kindling (Racine scale). When stage 5 seizures and tonic-clonic convulsions were fully developed, c-Fos expression was observed in the dentate gyrus [53]. This evidence suggests that strong neuronal stimulation is necessary to increase c-Fos in the hippocampus. Similarly, in our study, PTZ significantly increased c-Fos expression in hippocampal DG, CA1 and CA3 areas in accordance with our behavioural data. Furthermore, the number of c-Fos positive cells increased in CA1 area of hippocampus and decreased in DG and CA3 areas in SB-266970 administred rats. In addition, 5-CT decreased c-Fos expression in all hippocampal areas, but this data was not supported with our behavioral results. Due to the lack of data on c-Fos expression in the kindling model epilepsy of 5-CT in the literature, one study in acute PTZ-induced epilepsy model in mice, administration of SR-57227A, a 5-HT3 agonist, provided a significant decrease in seizure stage, but no significant decrease in c-Fos expression was observed in DG, CA1, CA3 and CA4 areas, showing that no correlation may not be found between behavioral data and c-Fos expression [54].

Conclusions

In conclusion, 5-HT7 antagonist SB-269970 showed anticonvulsant effects on PTZ-induced fully kindled rats. These anticonvulsive effects may be related to GABAergic neuronal activity in hippocampus. Further studies are needed for 5-HT7 antagonists to be used for therapeutic purposes in epileptic seizures.

Disclosure statement

No potential conflict of interest was reported by the author (s).

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ORCID

Bilal Sahin http://orcid.org/0000-0002-4419-1385
Ercan Ozdemir http://orcid.org/0000-0001-8231-1053
Erkan Gumus http://orcid.org/0000-0001-6432-7457
Mustafa Ergul http://orcid.org/0000-0003-4303-2996
Ahmet Sevki Taskiran http://orcid.org/0000-0002-5810-8415

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